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Population genetic analyses reveal distinct geographical blooms of the jellyfish *Rhizostoma octopus* (Scyphozoa)

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Understanding the spatial integrity and connectivity of jellyfish blooms is important for ecologists and coastal stakeholders alike. Previous studies have shown that the distribution of jellyfish blooms can display a marked consistency in space and time, suggesting that such patterns cannot be attributed to passive processes alone. In the present study, we have used a combination of microsatellite markers and mitochondrial COI sequences to investigate genetic structuring of the scyphozoan jellyfish *Rhizostoma octopus* in the Irish and Celtic Seas. The mitochondrial data indicated far higher levels of population differentiation than the microsatellites ($\Phi_{ST[MT]} = 0.300$ vs $\Phi_{ST[NUC]} = 0.013$). Simulation studies indicated that the low levels of nuclear differentiation were not due to limited power as a result of low levels of polymorphism. These findings, supported by palaeodistribution modelling and mismatch distribution analysis, are consistent with expansion of *R. octopus* from a single, limited refugium after the Last Glacial Maximum, followed by subsequent isolation, and that the discrepancy between the mitochondrial and nuclear markers is a result of the nuclear loci taking longer to reach mutation-drift equilibrium following the expansion due to their fourfold larger effective population size. The populations studied are most likely not well connected via gene flow, and thus genetically as well as geographically distinct, but our findings also highlight the need to use a combination of organellar and nuclear markers to give a more complete picture of population demography and structure, particularly for species with large effective population sizes.

ADDITIONAL KEYWORDS**:** Jellyfish, microsatellites, mitochondrial COI, palaeodistribution modelling, population genetics, *Rhizostoma octopus*

INTRODUCTION

The application of population genetics approaches has provided many insights into the levels and patterns of gene flow in marine organisms. Traditionally, it had been viewed that there were few barriers to population connectivity in the marine realm, particularly for organisms with planktonic or partially planktonic life cycles (Palumbi, 1994; Norris, 2000). Subsequent molecular studies on marine populations utilising mitochondrial DNA (mtDNA), however, indicated that intraspecific genetic structuring does exist (e.g. Chow *et al.,* 1997; Zane *et al.*, 1998; Keeney *et al.*, 2005; Darling, Kucera & Wade, 2007). More recently, the development of microsatellite markers has offered further opportunities to study genetic structuring, since theoretical studies have suggested that the use of multiple, multi-allelic loci should offer greater power than mtDNA to detect population subdivision, particularly at low levels (Larsson *et al.*, 2009), and this has been largely borne out by empirical studies (Iacchei *et al.*, 2014; Godhe *et al.*, 2014; but see Provan *et al.*, 2009). It has also been demonstrated, however, that population demographic changes such as those associated with the climatic fluctuations of the Pleistocene (*ca.* 2.58 MYA – 11 KYA) can give rise to apparently contradictory signals of population subdivision across different markers (Lukoschek, Waycott & Keogh, 2008; Larmuseau *et al.*, 2010). Thus, depending on the demographic history of the populations under study, the use of both mtDNA and microsatellites may be required to gain a complete picture of patterns of gene flow.

Within this context, there is international interest in the drivers, overall abundance and connectivity of jellyfish blooms (i.e. Phylum Cnidaria, Class Scyphozoa; Hamner & Dawson 2009; Brotz *et al.,* 2012; Condon *et al.,* 2013). These blooms represent the concentration of many free swimming medusae in a particular area either through rapid population growth (a true bloom) or advection from another area (an apparent bloom; Graham, Pag & Hamner,

2001; Graham *et al.,* 2003). True blooms are associated typically with species displaying metagenic life-histories comprising free-swimming and sexually reproducing medusae and benthic polyps that reproduce through asexual strobilation (e.g. Graham, Pag & Hamner, 2001; Richardson *et al.* 2009; Gibbons & Richardson 2013). In most cases, a given cohort of medusae will persist from spring through to autumn, whilst the asexually reproducing polyps can survive for many years (Thein, Ikeda & Uye, 2012). This inter-annual persistence of an asexually reproducing sessile life stage can lead to the regular re-occurrence of blooms in specific locations(Houghton *et al*., 2006a,b; Lilley *et al*., 2008), population structuring (e.g. Pitt & Kingsford, 2000) and eventual phylogenetic differentiation. As efforts to incorporate jellyfish more effectively into ecosystem and fisheries models gather momentum (Pauly *et al.* 2008; Brotz *et al.* 2012; Fleming *et al.* In Press), such information is important when considering the temporal and spatial integrity of seemingly isolated bloom events (Lee *et al.* 2013).

The utility of population genetics to elucidate the connectivity or discreteness of jellyfish blooms has been shown, with studies having revealed population structuring (Dawson, 2005a), cryptic speciation (Dawson & Jacobs, 2001; Holland *et al.*, 2004) and even anthropogenic introductions (Dawson, Gupta & England, 2005). Almost all such studies of scyphozoan jellyfish population genetics have employed a limited number of markers (with the exception of Aglieri *et al*. [2014]), with most studies relying mainly on the mitochondrial COI gene (eg Holland *et al*., 2004), although some have additionally employed data from the nuclear ribosomal DNA cistron (e.g. Dawson & Jacobs, 2001; Stopar *et al*., 2010). The development of microsatellite markers for several jellyfish species (Coughlan, Seymour & Cross 2006; Peplow *et al.* 2009; Reusch *et al.* 2010; Bolte *et al.* 2013; Meek *et al.* 2013), potentially offers the opportunity to study fine-scale genetic structuring, although to date, there has only been a single published study on scyphozoans (Aglieri *et al*. 2014).

In the present study, we used a combination of recently developed microsatellite markers and COI sequences to investigate genetic structuring of *Rhizostoma octopus*, a scyphozoan jellyfish with a generally predictable and temporally stable geographical distribution, including regular but apparently discrete blooms of adult jellyfish in bays in the Irish Sea (Doyle *et al.* 2006; Houghton 2006b). Previous genetic analyses within the genus have provided conflicting results, with Ramšak, Stopar & Malej (2012) finding little partitioning of genetic diversity between blooms of *R. pulmo* in the Mediterranean Sea, whilst Lee *et al.* (2013) found notable population structure in *R. octopus* in the Irish Sea and from La Rochelle, France, although levels of differentiation were far less pronounced in the nuclear gene studied (calmodulin) compared to the mitochondrial cytochrome oxidase subunit 1 (COI) gene. The use of microsatellites, with their potentially increased resolution, should allow us to determine whether any fine-scale structure exists in *R. octopus*, even in cases where such levels may be extremely low (Wirth & Bernatchez, 2001), but also whether there are any discrepancies between mtDNA and microsatellites, possibly resulting from demographic changes during the Pleistocene.

MATERIALS AND METHODS

SAMPLING AND DNA EXTRACTION

Samples were collected from four locations throughout the Irish and Celtic Seas (Table 1 and Figure 1) in August / September 2011. Genomic DNA was extracted using a modified version of the Porebski, Bailey & Baum (1997) CTAB phenol/chloroform protocol whereby extracted DNA which had been subjected to phenol and chloroform wash was stored in a 1:1 supernatant:isopropanol state at -20°C until needed for PCR, then pelleting and the alcohol wash were carried out before elution. Long term storage of eluted DNA resulted in loss of high molecular weight (genomic) DNA and reduced amplification success.

MICROSATELLITE GENOTYPING

Microsatellites were developed from *R. pulmo* sequences deposited in GenBank (for accession numbers see Table 2). Forward primers included a 19 bp M13 tail (CACGACGTTGTAAAACGAC) and reverse primers included a 7 bp tail (GTGTCTT). PCR was carried out in a total volume of 10 µl containing 100 ng genomic DNA, 10 pmol of 6-FAM- or HEX-labelled M13 primer, 1 pmol of tailed forward primer, 10 pmol reverse primer, 1x PCR reaction buffer, 200 µM each dNTP, 2.5 mM $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase (Promega). PCR was carried out on a MWG Primus thermal cycler using the following parameters: initial denaturation at 94 °C for 5 min followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s (55 °C for RpMS-4), extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping system (Life Technologies; Carlsbad, California, USA). Allele sizes were scored using LIZ size standards and were checked by comparison with previously sized control samples.

MTDNA SEQUENCING

A 639 bp region of the *R. octopus* mtDNA COI gene was amplified using the primers Ro-COI-F 5'-CAACAAATTCTAAGATATTGGAAC-3' and Ro-COI-R 5'-GGGTCGAAGGAAGATGTATTA-3'. PCR was carried out on a MWG Primus thermal cycler using the following parameters: initial denaturation at 94 °C for 3 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 20 µl containing 200 ng genomic DNA, 10 pmol of each primer, 1x PCR reaction buffer, 200 µM each dNTP, 2.5 mM $MgCl_2$ and 0.5 U GoTaq Flexi DNA polymerase (Promega). 5 µl PCR product were resolved on 1.5% agarose gels and visualised by ethidium bromide staining, and the remaining 15 µl were EXO-SAP purified and sequenced in both directions using the BigDye sequencing kit (V3.1; Applied Biosystems) and run on an AB 3730XL DNA analyser (Life Technologies; Carlsbad, California, USA).

DATA ANALYSIS

Tests for linkage disequilibrium between pairs of microsatellite loci in each population were carried out in the program FSTAT (V2.9.3.2; Goudet, [2002]). Levels of polymorphism measured as observed (H_O) and expected (H_E) heterozygosity averaged over loci for nuclear microsatellites, and as haplotype (H) and nucleotide (π) diversity for mtDNA, were calculated using the ARLEQUIN software package (V3.5.1.2; Excoffier & Lischer, [2010]). Inbreeding coefficients (F_{IS}) were estimated using FSTAT. Levels of interpopulation differentiation were estimated from allele (microsatellite) and haplotype (mtDNA) frequencies using Φ-statistics, which give an analogue of F-statistics (Weir & Cockerham, 1985) calculated within the analysis of molecular variance (AMOVA) framework (Excoffier, Smouse & Quattro 1992), also using the ARLEQUIN software package. Population-pairwise Φ_{ST} values were also

138 calculated using ARLEQUIN. Significance of values was tested using 1,000 permutations. A
139 median-joining network showing the relationships between the mtDNA haplotypes was
140 constructed using the NETWORK software package (V4.5.1.6; www.fluxus-engineering.com).
141 In addition, tests for population expansion based on Tajima's *D* and Fu's F_S and a mismatch
142 distribution analysis, which identifies characteristic "waves" in the shape of the distribution
143 resulting from expansion (Rogers and Harpending, 1992), were carried out in ARLEQUIN.
144 To identify possible spatial patterns of gene flow, the software package BAPS (V5;
145 Corander, Waldmann & Sillanpää, [2003]) was used to identify clusters of genetically similar
146 populations using a Bayesian approach. Ten replicates were run for all possible values of the
147 maximum number of clusters (*K*) up to $K = 4$, the number of populations sampled in the
148 study, with a burn-in period of 10 000 iterations followed by 50 000 iterations. Multiple
149 independent runs always gave the same outcome. To further identify possible spatial patterns
150 of gene flow, a principal coordinate analysis (PCoA) was carried out in GENALEX (V6.1;
151 Peakall & Smouse, 2006). Inter-individual genetic distances were calculated as described in
152 Smouse & Peakall, 1999, and the PCoA was carried out using the standard covariance
153 approach.
154 Because of the genetic homogeneity revealed by the microsatellite loci studied, and to
155 compare the relative power of microsatellites and the mtDNA to detect low levels of
156 population differentiation, simulations were carried out using the POWSIM software package
157 (V4.0; Ryman & Palm, 2006). Simulations were carried out for an effective population size
158 of $N_e = 1\ 000$ to yield F_{ST} values of 0.0050, 0.0075, 0.0100, 0.0125, 0.0150, 0.0175 and
159 0.0200. Although *R. octopus* may have a larger effective population size, this is not relevant
160 to the analysis, since N_e only determines the time necessary to reach the target F_{ST}. Thus, the
161 use of larger values of N_e is unjustified as the difference between, say, $N_e = 1\ 000$ and 10 000
162 (and higher) is not important at values of F_{ST} as small as those tested in the simulation (Nils

163 Ryman, personal communication). In all cases, 1 000 replicates were run and the power of
164 the analysis was indicated by the proportion of tests that were significant at $P < 0.05$ using
165 the observed allele frequencies for both the four microsatellite loci and the single mtDNA
166 COI region studied (for $F_{ST} = 0$ this corresponds to the Type I [α] error). For the mtDNA,
167 sample sizes were adjusted as recommended by Larsson *et al.*, (2009).
168
169 PALAEODISTRIBUTION MODELLING
170 Palaeodistribution modelling was carried out to determine the potential suitable range for *R.*
171 *octopus* at the Last Glacial Maximum (LGM; *ca*. 21 KYA) using the maximum entropy
172 approach implemented in the MAXENT software package (V3.3.3; Phillips, Anderson &
173 Schapire, 2006). Species occurrence data between 1950 and 2000 were downloaded from the
174 Global Biodiversity Information Facility data portal (www.gbif.org) and from the Ocean
175 Biogeographic Information System (www.iobis.org), and supplemented with our own
176 population data from the current study (117 spatially unique occurrences in total). Current-
177 day bioclimatic data (MARSPEC; Sbrocco & Barber, 2013) were obtained at 5 minute
178 resolution and models were generated using cross-validation of ten replicate runs under the
179 default MAXENT parameters. Model performance was assessed based on the area under the
180 receiver operating characteristic curve (AUC). Models were projected onto reconstructed
181 bioclimatic data for the LGM (ensemble of five models: CNRM, ECBILTCLIO, FGOALS,
182 HadCM and MIROC-322; Sbrocco, 2014). To identify potential areas where the model may
183 have extrapolated beyond current climatic conditions, which could lead to unreliable
184 predictions, we carried out a multivariate environmental similarity surfaces (MESS) analysis
185 (Elith *et al.* 2010) in MAXENT.

RESULTS

POPULATION GENETIC ANALYSES

No evidence of linkage disequilibrium was detected between any of the four nuclear microsatellite loci analysed. Between 13 (Rp-MS1) and 25 (Rp-MS5) alleles were detected, with a total of 73 (mean = 18.25 per locus). Within-population levels of observed (H_O) and expected (H_E) heterozygosity ranged from 0.658 (Solway Firth) to 0.777 (Carmarthern Bay; mean = 0.729) and from 0.805 (Tremadoc Bay) to 0.852 (Carmarthen Bay; mean = 0.822) respectively (Table 1). Levels of F_{IS} were significantly different from zero in three of the four populations, and ranged from 0.074 (Tremadoc Bay) to 0.188 (Solway Firth; mean = 0.075). Summary statistics by locus are given in Supplementary Table S1.

A total of 27 mitochondrial COI haplotypes were identified (Figure 2). Nineteen of these were found in a single individual, and three of the remaining eight, including the two most common haplotypes, were found in more than one population. Within populations, between three (Tremadoc Bay) and 15 (Carmarthen Bay) haplotypes were detected (mean = 8.25). Levels of haplotype (H) and nucleotide (π) diversity ranged from 0.178 (Tremadoc Bay) to 0.920 (Carmarthen Bay), and from 0.001 (Tremadoc Bay) to 0.006 (Solway Firth) respectively (Table 1).

The analysis of molecular variance (AMOVA) revealed a small but significant overall differentiation based on nuclear microsatellites ($\Phi_{ST[NUC]} = 0.013$; $P < 0.001$), and a much higher level based on the mtDNA COI ($\Phi_{ST[MT]} = 0.300$; $P < 0.001$; Table 3). Population-pairwise Φ_{ST} values ranged from zero (three pairs) to 0.046 (Tremadoc Bay / Celtic Sea) for nuclear microsatellites, and from zero (Carmarthen Bay / Celtic Sea) to 0.579 (Tremadoc Bay / Celtic Sea) for the mtDNA COI (Table 4). The BAPS analysis indicated that all the individuals analysed were grouped into a single genetic cluster (100% probability). This was

211 reflected in the PCoA, which showed no evidence of geographical structuring of individual
212 multilocus genotypes (Figure 3). The values for both Tajima's *D* and Fu's F_S were
213 significantly negative (-1.434 [$P = 0.049$] and -16.077 [$P < 0.0001$] respectively), consistent
214 with sudden population expansion. The mismatch distribution analysis (Figure S1), which
215 resulted in a Harpending's raggedness index of 0.045 ($P = 0.297$), also did not reject the
216 sudden expansion model.
217 The simulation studies suggested that the nuclear microsatellite data were able to detect
218 F_{ST} values of as low as 0.0100 at least 90% of the time, and 0.0125 at least 98% of the time
219 (Figure 4). The mtDNA COI locus had much lower power, only 9% and 16% for the same
220 two values, and could only detect $F_{ST} = 0.05$ in 88% of the simulations. At the lowest values
221 of F_{ST} (≤ 0.01) used in the simulations, the power of the nuclear microsatellite loci was
222 generally five- to ten-fold that of the mtDNA COI locus.
223
224 PALAEODISTRIBUTION MODELLING
225 For all models, AUC values were high (mean AUC = 0.995). The modelled current-day
226 distribution was a largely accurate prediction of the current range of *R. octopus*, highlighting
227 coastal areas of northwestern Europe as most suitable (Figure 5a). The palaeodistribution
228 model indicated extensive suitable habitat in the Mediterranean at the LGM, but very little in
229 the northeast Atlantic, with the only suitable habitat being limited to a small area in the Bay
230 of Biscay adjacent to the palaeocoastline (Figure 5b). The MESS analysis did not indicate
231 any areas in the model where extrapolation beyond current climatic conditions had occurred.

DISCUSSION

Although the results from the two sets of markers in the present study revealed differing levels of population structuring, they can be interpreted as being generally consistent with population expansion following the LGM and subsequent divergence, with limited gene flow between the regions studied. Our findings are broadly comparable with those from a previous study on *R. octopus* (Lee *et al.* 2013), and highlight an emerging trend from the currently limited number of microsatellite-based population genetic analyses in gelatinous zooplankton (Bolte *et al.*, 2013; Aglieri *et al.*, 2014), namely that blooms can readily be traced to relatively isolated, self-sustaining populations. From an ecological perspective such information is insightful given that scyphozoa have often been viewed as transient components of marine food webs, with very little spatial integrity or trophic relevance (Doyle *et al.,* 2006; Houghton *et al.,* 2007). The growing body of evidence to show that jellyfish blooms can persist in large numbers in particular locations over time (through processes in addition to advection) promotes the much needed inclusion of such species in ecosystem models (Pauly *et al.,* 2008; Doyle *et al.,* 2014).

Discrepancies between the levels of genetic structuring revealed by nuclear and organellar markers have been reported in a wide range of species (reviewed in Karl *et al.* 2012). These can be the result of a variety of processes, including sex-biased dispersal (Cano, Mäkinen & Merilä, 2008), homoplasy at microsatellite loci (Estoup, Jarne & Cornuet, 2002), selection (de Innocentiis *et al.,* 2001), or differences in effective population size (Paulmbi, Cipriano & Hare, 2001). The observed disparity between levels of population differentiation revealed by nuclear and mitochondrial markers in the present study, which differ by more than an order of magnitude ($\Phi_{ST[\mathrm{NUC}]} = 0.013$ vs. $\Phi_{ST[\mathrm{MT}]} = 0.30$), can be explained most readily by the last of these. For diploid species, such as *R. octopus*, the effective population size of the haploid

257 mitochondrial genome is half that of the diploid nuclear genome. In addition to this, in
258 idealized populations of dioecious taxa with even sex ratios, the effective population size of
259 the mitochondrial genome could be assumed to be 0.25 of the effective population size of the
260 nuclear genome, leading to differences in the time required for reciprocal monophyly via
261 lineage sorting (Maynard Smith 1987; Paulmbi, Cipriano & Hare 2001; Hudson & Coyne
262 2002). A lack of resolving power due to insufficient polymorphism in the microsatellites is
263 not supported by the simulation analyses, which indicated that the microsatellites had far
264 greater power than mtDNA over a range of simulated F_{ST} values based on the empirical allele
265 frequencies.

266 Differences in F_{ST} and its equivalents between nuclear and mitochondrial markers can be
267 further exaggerated where populations have undergone recent expansion. In such
268 circumstances, nuclear loci will take longer to reach mutation-drift equilibrium. This has
269 been suggested previously for other marine species with large effective population sizes
270 (Lukoschek, Waycott & Keogh, 2008; Larmuseau *et al.*, 2010). The results of the
271 palaeodistribution modelling indicate an extremely restricted area of suitable habitat for *R.*
272 *octopus* in the northeast North Atlantic during the LGM compared to its current distribution.
273 The model did suggest the presence of suitable habitat in the Mediterranean, but whilst this
274 area was not isolated from the Atlantic despite the drop in sea levels during the glacial period,
275 the Strait of Gibraltar represents a major biogeographic barrier to a range of marine species
276 (Baus, Darrock & Bruford 2005 and references therein; Paternello, Volckaert & Castilho
277 2007). Furthermore, climate-induced range shifts and contractions such as those that
278 occurred during the Pleistocene are believed to result from population extirpation, rather than
279 migration (Dalén *et al.* 2007; Bennett & Provan 2008; Provan & Bennett 2008). Our
280 findings, including the significant negative values for both Tajima's D and Fu's F_S and the
281 mismatch distribution analysis, are consistent with expansion of *R. octopus* from a single,

limited refugium after the LGM, followed by subsequent isolation, as indicated by the mtDNA and the nuclear F_{IS} values, which suggest inbreeding within three of the four populations. Many northern North Atlantic marine species survived the LGM in a range of refugia (reviewed in Provan 2013), and the low levels of nuclear genetic differentiation observed in *R. octopus* are consistent with high historical gene flow, suggesting an extended period of genetic connectivity consistent with LGM survival of populations in the same area. Population isolation following the expansion would give rise to the observed discordance between mtDNA and microsatellites.

Despite the discrepancies observed between mtDNA and microsatellites, the case for using multiple, unlinked nuclear loci for genetic studies on scyphozoa is strong. As a basic tool, the mitochondrial COI marker allows a great deal of information to be gathered and comparisons to be made with many other scyphozoan species for which population data sets exist (e.g. Dawson, 2005; Holland *et al*., 2004; Prieto, Armani & Marcias, 2013). The additional potential power of microsatellites, as indicated by the simulation studies, could be useful in fine-scale analyses of population structure in other species which appear to have little geographically-based population structuring such as the congener, *R. pulmo* (Ramšak *et al*., 2012). With the recent publication of a study of *Pelagia noctiluca* genetics employing microsatellite markers (Aglieri *et al*., 2014) and the present study, we foresee a shift in scyophozoan studies toward including panels of unlinked, high-resolution nuclear markers. As in the present study, a combination of organellar and nuclear markers may be necessary to give a more complete picture of population demography and structure, particularly for species with large effective population sizes.

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Table 1. *Rhizostoma octopus* populations studied and summary diversity statistics

Population	Latitude (N)	Longitude (W)	Nuclear				Mitochondrial			
			N	H_O	H_E	F_{IS}	*N*	*h*	*H*	π
Carmarthern Bay	51.745	4.447	24	0.777	0.852	0.090^{**}	24	15	0.920	0.004
Tremadoc Bay	52.728	4.066	23	0.765	0.824	0.074^{NS}	22	3	0.178	0.001
Solway Firth	54.958	3.217	24	0.658	0.805	0.188^{**}	19	10	0.854	0.006
Celtic Sea	51.783	6.650	15	0.717	0.808	0.117^{*}	14	5	0.659	0.003

Abbreviations: *N*, number of individuals studied; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; *h*, number of haplotypes detected; *H*, gene diversity; π, nucleotide diversity. Significance of F_{IS} - * $P < 0.05$; ** $P < 0.01$; *NS* – non-significant.

Table 2. *Rhizostoma octopus* microsatellite primers

Locus	Repeat	Primers (5' – 3')	Size range (bp)	GenBank
Rp-MS1	$(GCACGCACACAC)_7$	F: CCCTCATACGTTATGTCATGG	148-205	DQ093644
		R: CAGCAGTTCTGACAAGTATTTATTATTC		
Rp-MS3	$(TGX)_{14}$	F: TTTGGTCGTGTCCTGTTTGA	141-212	DQ075948
		R: CGCCAAGAGCAGAATCAATA		
Rp-MS4	(ACTACAC) complex	F: CCAACTAATAGAAACTAATCTAGACTAAAC	398-467	DQ075951
		R: AAAGTATGATTACGTGAAACGA		
Rp-MS5	(TACAC) complex	F: AAAATTTGCTCTTATTTGATTCTCG	237-362	DQ075950
		R: GATGAAAATCGTGGAAGCTG		

Forward tailed with CACGACGTTGTAAAACGAC

Reverse tailed with GTGTCTT

Table 3. Analysis of molecular variance (AMOVA)

Source of variation	Nuclear				Mitochondrial			
	d.f	Sum of squares	Variance	%	d.f	Sum of squares	Variance	%
Among populations	3	6.666	0.019	1.33***	3	9.153	0.140	30.02***
Within populations	168	236.979	1.411	98.67	75	24.417	0.326	69.98

*** $P < 0.001$

Table 4. Population-pairwise ST values. Lower diagonal matrix – nuclear; Upper diagonal matrix – mitochondrial. Values not significantly different from zero are shown in italics.

Carmarthen Bay	-	0.437	0.100	*0.068*
Tremadoc Bay	*-0.005*	-	0.410	0.579
Solway Firth	*-0.011*	0.039	-	0.206
Celtic Sea	0.029	0.046	*-0.006*	-
	Carmarthen Bay	Tremadoc Bay	Solway Firth	Celtic Sea

Figure Legends

Figure 1. Locations of sites sampled in this study: CB – Carmarthen Bay; TB – Tremadoc Bay; SF – Solway Firth; CS – Celtic Sea. Inset map shows western Europe, highlighting the area of the present study.

Figure 2. Median-joining network showing relationships between the 27 haplotypes detected by sequencing the mtDNA COI region. Circle sizes are approximately proportional to haplotype frequency: smallest circle represents a single individual, largest circle represents 24 individuals. Each connection represents a single mutation and small open diamonds represent missing intermediate haplotypes.

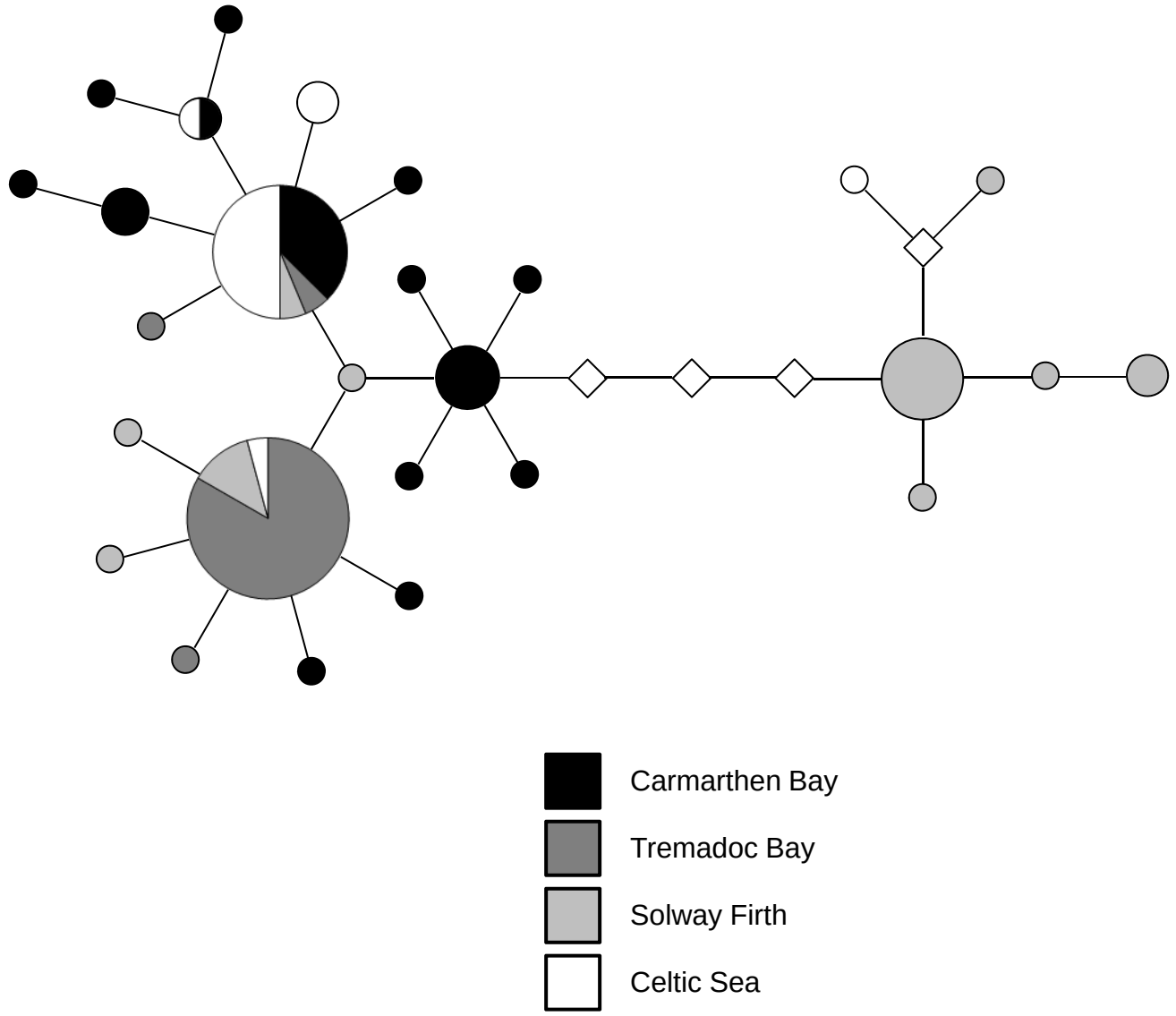
Figure 3. Results of the PCA. The first three axes accounted for 23.51%, 21.54% and 17.44% respectively of the total variation (62.49%).

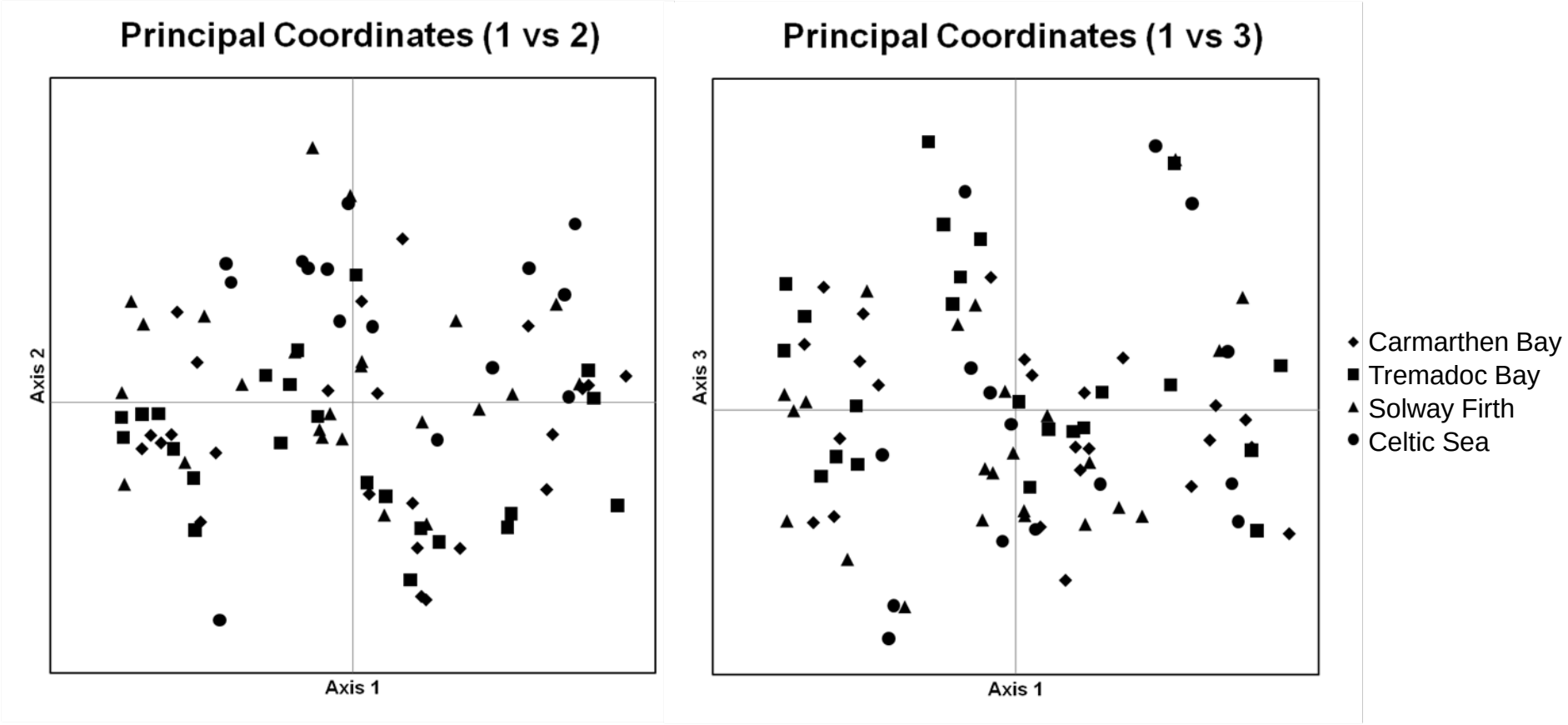
Figure 4. Results of the POWSIM analysis. The Y-axis represents the power of the markers to successfully recover the value of F_{ST} indicated on the X-axis, expressed as the proportion of 1000 simulations (see text for details). For $F_{ST} = 0$, this is the Type I (α) value.

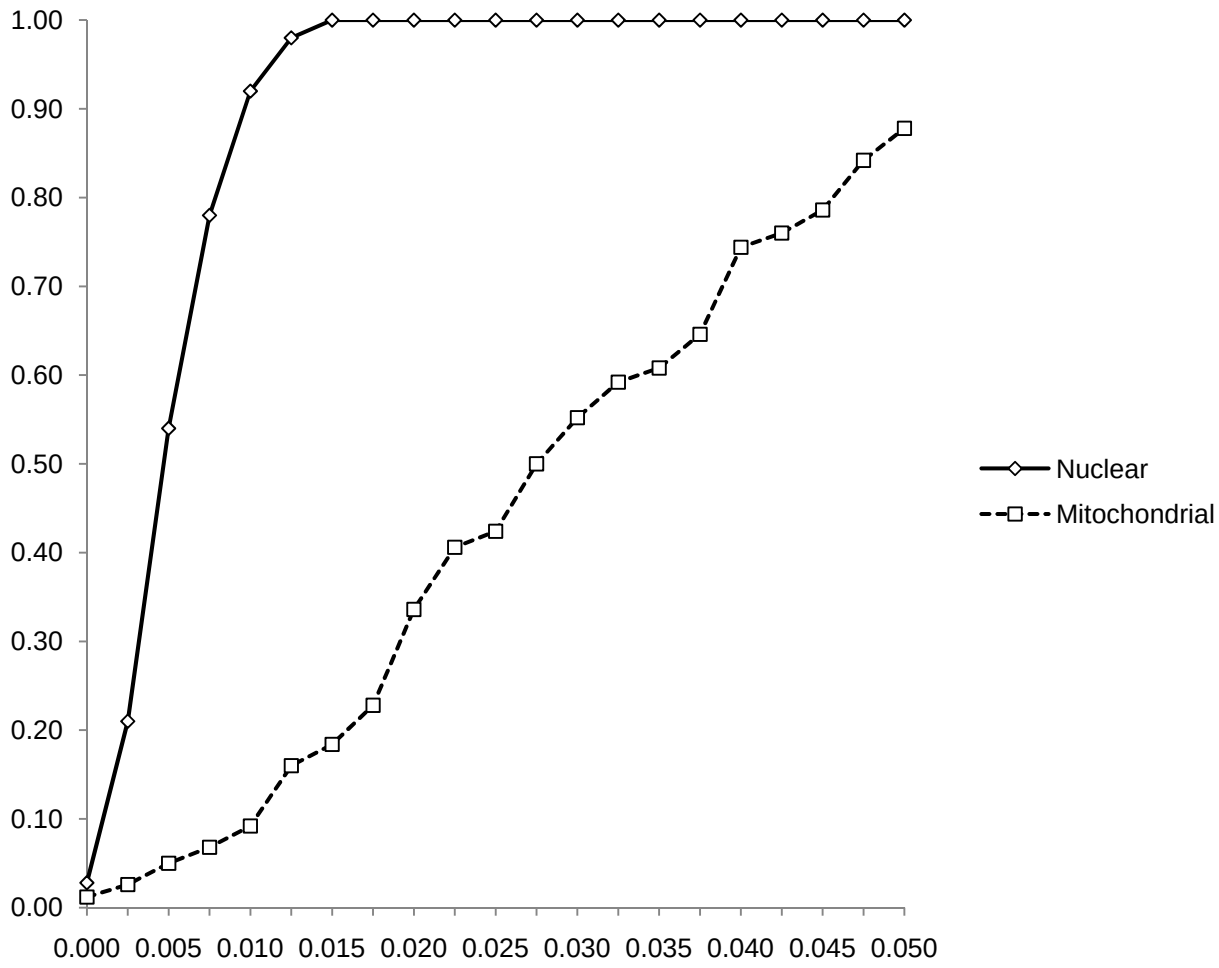
Figure 5. Results of the species distribution modelling: (a) current-day model; (b) palaeodistribution model for the Last Glacial Maximum (LGM *ca.* 21 KYA). Darker blue areas indicate those more suitable for *R. octopus*. Yellow circles in (a) indicate occurrence data used to generate the models.

Figure S1. Results of the mismatch distribution analysis.


Scotland
SF
Ireland
TB
Wales
CS
CB
0
50
100
km


Carmarthen Bay
Tremadoc Bay
Solway Firth
Celtic Sea


Principal Coordinates (1 vs 2)
Axis 2
Axis 1
Principal Coordinates (1 vs 3)
Axis 3
Axis 1
Carmarthen Bay
Tremadoc Bay
Solway Firth
Celtic Sea


1.00
0.90
0.80
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0.50
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0.005
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0.020
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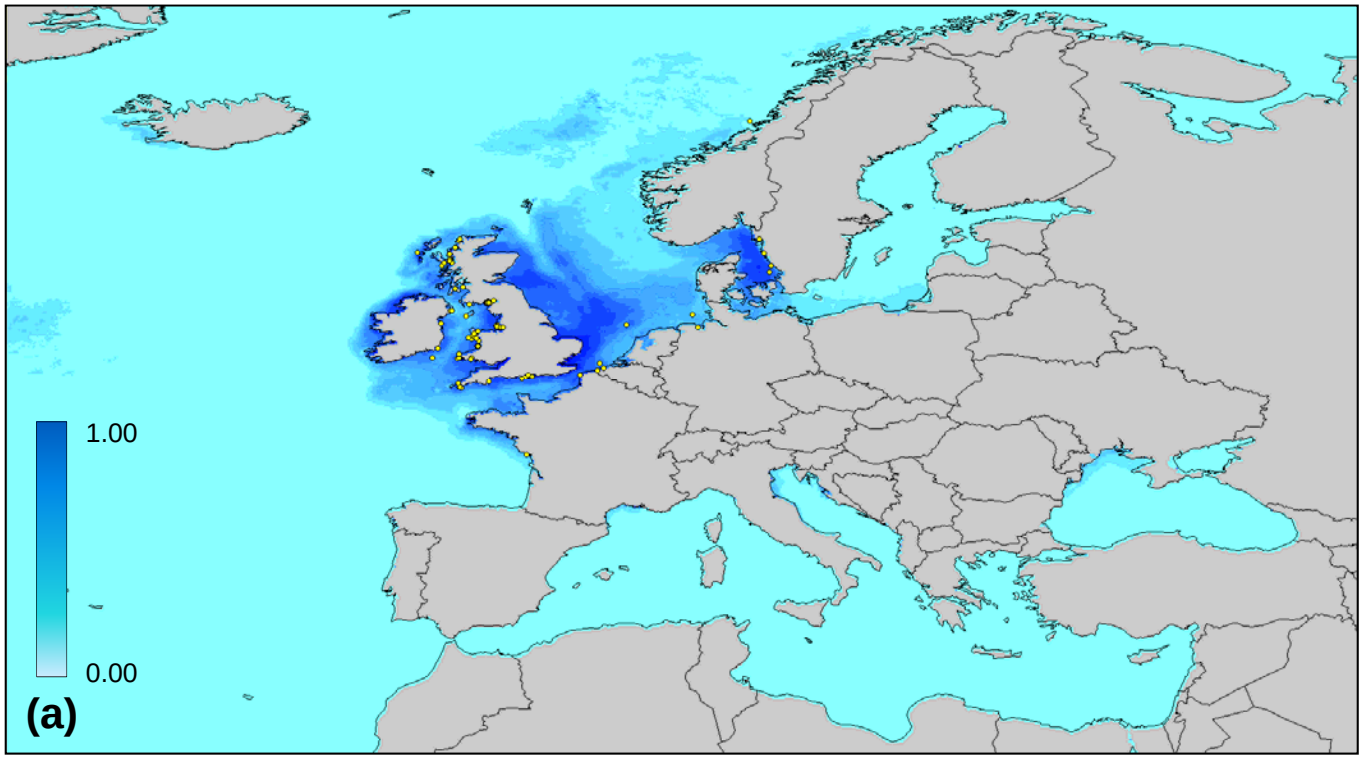
0.040
0.045
0.050
Nuclear
Mitochondrial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(a)

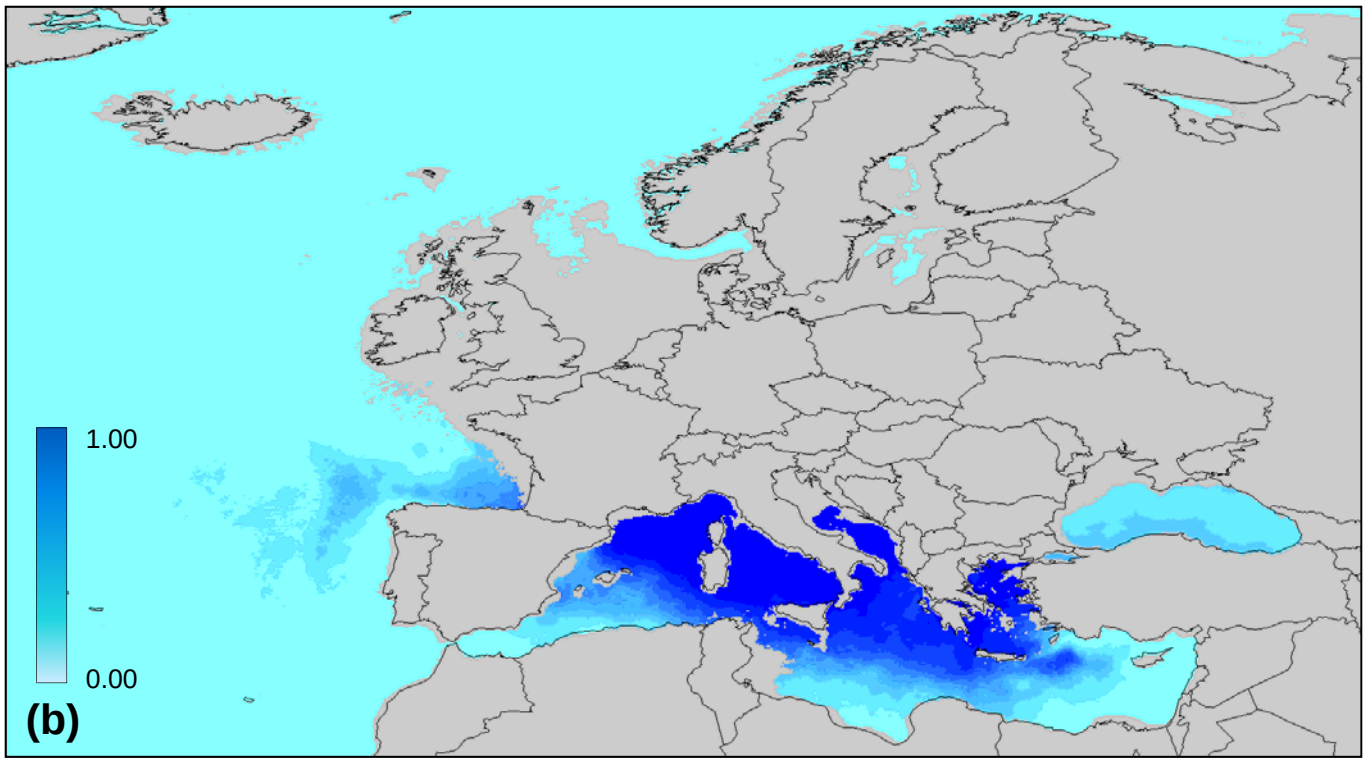
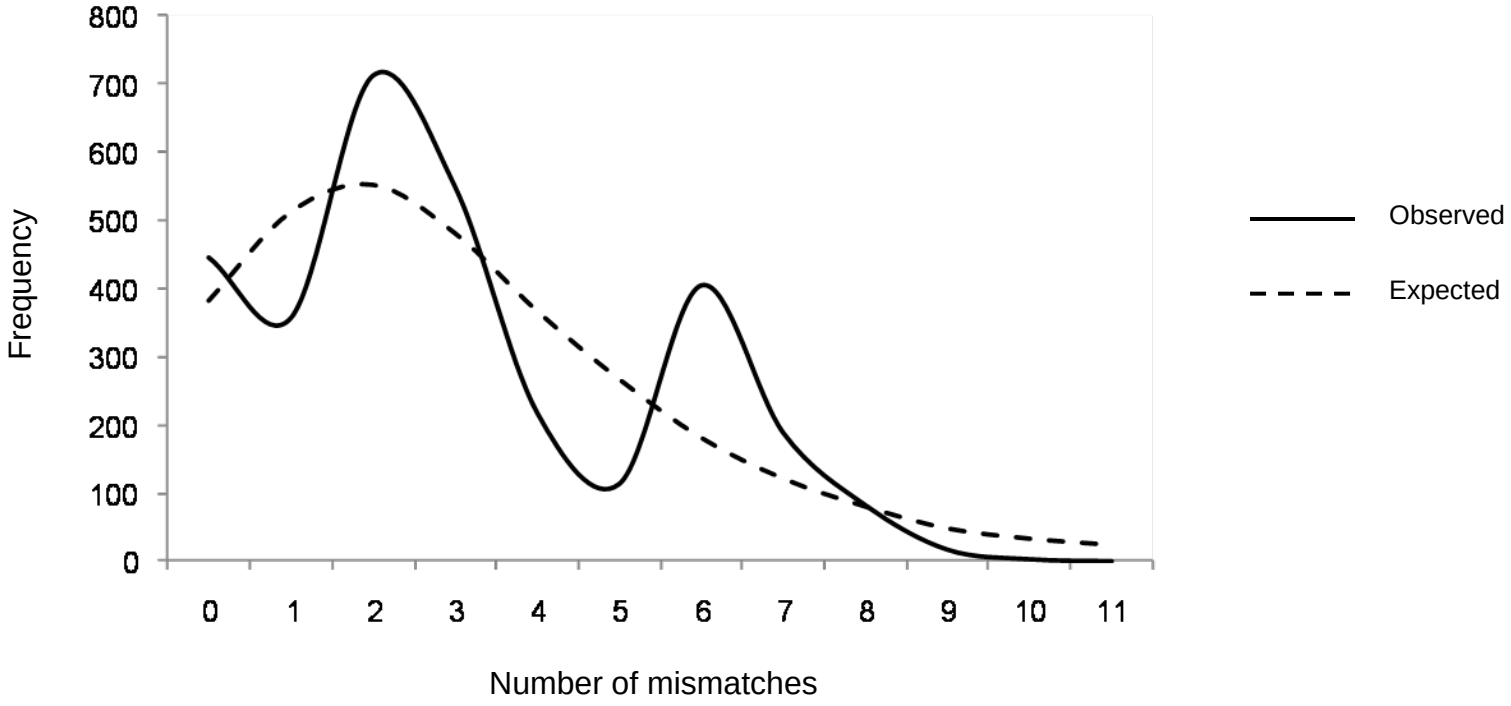

1.00
0.00
(b)

Table S1 Summary statistics by locus. Abbreviations: *N*, number of individuals studied; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient.

Locus		Population			
		Carmarthen Bay	Tremadoc Bay	Solway Firth	Celtic Sea
		N =24	*N* = 23	*N* = 24	*N* = 15
Rp-MS1	H_O	0.565	0.571	0.591	0.733
	H_E	0.685	0.650	0.629	0.784
	F_{IS}	0.178	0.124	0.062	0.067
Rp-MS3	H_O	0.833	0.818	0.750	0.667
	H_E	0.910	0.886	0.876	0.851
	F_{IS}	0.085	0.078	0.146	0.222
Rp-MS4	H_O	0.833	0.905	0.789	0.667
	H_E	0.861	0.816	0.797	0.641
	F_{IS}	0.033	-0.111	0.009	-0.041
Rp-MS5	H_O	0.875	0.765	0.500	0.800
	H_E	0.953	0.945	0.919	0.956
	F_{IS}	0.083	0.195	0.464	0.168


800
700
600
500
400
300
200
100
0
Frequency
0
1
2
3
4
5
6
7
8
9
10
11
Number of mismatches
Observed
Expected